with 1, 2, 3, or 4 m-moles/kg. Experiments in 40 mice with histidine at 4 m-moles/kg also were negative. The following substances tested at 3 m-moles/kg in the indicated number of mice also produced none of the effects noted with IMA at this level: imidazole, 10; 4-hydroxymethylimidazole, 10; 1-methylimidazole-4-acetic acid, 7; imidazolepyruvic acid, 10; imidazoleacrylic acid, 10; 1-acetylimidazole, 10; 4-imidazole carboxylic acid, 10; and 4,5-imidazole dicarboxylic acid, 10. Therefore,

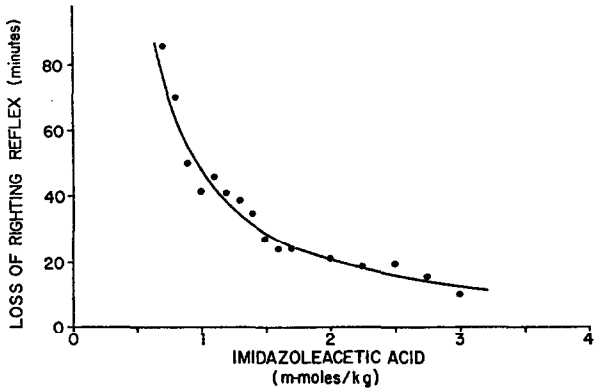

FIG. 2. Average times required for loss of righting reflex as a function of dose of imidazoleacetic acid.

it appears that considerable structural specificity must be associated with the biological effects of imidazoleacetic acid in mice.

Models show that in IMA the carboxyl group could form a hydrogen bond with the ring nitrogen in the 3-position. The molecule then could appear like a fused ring system, one of the surfaces of which is almost planar. Studies are under way of various physical parameters which might be related to the physiological effects of IMA and of the distribution, metabolism, and biochemical effects of the substance.

Department of Biochemistry,
City of Hope Medical Center,
Duarte, Calif., U.S.A.

EUGENE ROBERTS
DAISY G. SIMONSEN

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Blocking effect of puromycin, ethanol, and chloroform on the development of tolerance to an opiate*

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PUROMYCIN has been found to block retention of a conditioned avoidance response,[1] suggesting that this learning process is associated with protein formation. Recently Cohen *et al.*[2] found that tolerance

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to the analgetic action of morphine was partially prevented by the prior administration of actinomycin and suggested that tolerance to morphine might represent some form of memory.

It has been observed[3] that tolerance developed to the lenticular effects of levorphanol or morphine. This response in the mouse was quantitatively represented by a parallel shift to the right of the dose–effect curve. The lenticular response in rodents was first described by Weinstock and her co-workers,[4] who found that parenteral injection of opiates produced a transient lenticular opacity which appeared to be restricted to opiates and could be blocked by narcotic antagonists. Because the ED_{50} for the lenticular effect of a large number of opiates paralleled the ED_{50} for analgesia, Weinstock[5] suggested that the receptors for analgesia and for production of lenticular opacities might be similar. It was reported recently[6] that actinomycin or puromycin given intraperitoneally prevented the development of long-term but not acute tolerance to the lenticular action of a subsequent dose of levorphanol. These antibiotics mainly affected protein synthesis,[7] so that tolerance to the lenticular actions of levorphanol appeared to be associated with formation of a new protein. The brain was regarded as the probable site for initiating these complex events, a view which was supported by the finding[8] that lenticular opacities can be produced by intracerebral injection of levorphanol in doses that are parenterally ineffective. Furthermore, studies with levorphanol-H^3 injected intracerebrally revealed that in comparison with the similar concentrations in the cerebral hemispheres and brain stem there was little radioactivity in the lens or in the plasma. The last observation suggested that the injected levorphanol was transported to other parts of the brain by the cerebral fluid rather than the blood.

In the hope of blocking tolerance, puromycin was injected intracerebrally just prior to the parenteral dose of levorphanol. The next day each mouse was injected with a large dose (ED_{80}) of levorphanol. Their eyes were inspected for opacities every 15 min during the next 2 hr, as previously described.[3] The results of this study with appropriate controls are listed in Table 1 and show that intracerebral puromycin significantly inhibited the development of lenticular tolerance.

TABLE 1. EFFECT OF INTRACEREBRAL INJECTION OF PUROMYCIN

Pretreatment at 24 hr	Response to levorphanol, i.p. (150 μmoles/kg)		
	No. opaque/ No. survivors	Deaths	Incidence (%)
None	72/90	1	80
Levorphanol, 75 μmoles/kg s.c.	13/59	0	22
Normal saline i.c. and levorphanol, 75 μmoles/kg s.c.	5/24	0	20
Puromycin, 4 mg/kg i.c. and levorphanol, 75 μmoles/kg s.c.	13/20*	2	65

* $P < 0{\cdot}01$ compared with control injection of normal saline.

Female Swiss-Webster mice weighing 20–25 g, the dose of 4 mg/kg body weight was injected into the right cerebrum (i.c.) in a volume of 30 μl with a 30-gauge needle and a microsyringe (Hamilton). The injection was given 10 min before levorphanol (75 μmoles/kg) administered subcutaneously (s.c.). This dose ordinarily produces a significant tolerance. The next day an ED_{80} of levorphanol (150 μmoles/kg) was injected i.p.

Because of the presumed central origin of tolerance to the lenticular effects, it was speculated that substances with anesthetic properties might influence tolerance development. As shown in Table 2, ethanol given as a 25% solution in normal saline (v/v) in a dose of 4 g/kg i.p. virtually prevented the development of tolerance to levorphanol (75 μmoles/kg). Chloroform was also effective. Chloroform anesthesia was initiated just prior to levorphanol administration and was maintained for the next 2 hr by an open technique. During this period air was continuously mixed with vapors to minimize hypoxia. In contrast, large doses of pentobarbital, chlorpromazine, or atropine did not prevent the development of tolerance to concurrently administered levorphanol. The data also show that the

administration (24 hr earlier) of ethanol without levorphanol did not affect the subsequent response to an ED_{80} of levorphanol. Actinomycin given in a large dose of 1 mg/kg i.p. partially blocked tolerance development as did intracerebral puromycin. When administered intraperitoneally, the dose of puromycin needed to block tolerance was 40 mg/kg.

Because the process of lenticular opacification induced by opiates is still unknown, the mechanism by which ethanol and chloroform block the development of lenticular tolerance can only be guessed. That the brain seems to initiate the lenticular opacity is suggested by studies[8] with intracerebral levorphanol. Tolerance would also seem to depend on some central mechanism involving protein formation, because the injection of a relatively small dose of puromycin (4 mg/kg) into the brain blocks its development. Because both ethanol and chloroform have a profound effect on the function of the central nervous system, it is reasonable to consider that these compounds block tolerance

TABLE 2. THE EFFECT OF SOME CENTRALLY ACTIVE SUBSTANCES ON THE DEVELOPMENT OF TOLERANCE TO LEVORPHANOL

Drug	No. opacities/ No. survivors	Deaths	Incidence (%)
None	11/54	1	20
Chlorpromazine	1/25	0	4
Pentobarbital (50 mg/kg)	10/73	23	14
Atropine (5 mg/kg)	2/22	3	9
Actinomycin (1 mg/kg)*	15/26	2	58†
Ethanol (4 g/kg)	20/29	1	69†
Ethanol (4 g/kg)‡	20/25	0	80
$CHCl_3$§	34/66	9	51†

The drugs were administered i.p. 10 min before the injection s.c. of levorphanol (75 μmoles/kg) except for actinomycin which was given 1 hr earlier; 24 hr later levorphanol was injected i.p. in a dose of 150 μmoles/kg (ED_{80}). The responders and those animals surviving 2 or more hr are listed along with the number of deaths that occurred over the total period. The incidence of opacity among survivors is expressed in percentages.

* Mice were sick the next day.

† Significantly different from the control $P < 0{\cdot}01$ calculated by the X^2 method.

‡ Levorphanol pretreatment (75 μmoles/kg) was not given.

§ Administered as the vapor.

development by interfering with some activity of the nervous system. Whether nerve transmission is interfered with or a direct action of these agents on synthesis of the protein is involved, or whether there are separate causes, cannot be decided from the present data.

Departments of Anesthesiology and Medicine,
New York Medical College,
New York, N.Y., U.S.A.

ALFRED A. SMITH
META KARMIN
JOANN GAVITT

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